after 30 years of detector development, only the evaporative light-scattering detector has offered any viable alternative. As a consequence, the future of the refractive index detector for specific applications still appears assured. In most applications an isocratic development procedure can be found that will provide satisfactory resolution of those solutes of interest, and so the problem of gradient elution can be circumvented. In addition, by using columns of 4.6 mm i.d. or more, the limited sensitivity of the refractive index detector can also be accommodated.

Further Reading

Parriott D (1993) *A Practical Guide to HPLC Detection*. San Diego: Academic Press.

Patonay G (1992) *HPLC Detector*. Newer Methods. New York: VCH.

Scott RPW (1996) *Chromatography Detectors*. New York: Marcel Dekker.

Vickrey TM (ed.) (1983) *Liquid Chromatography Detectors*. New York: Marcel Dekker.

Yeung ES (ed.) (1986) *Detectors for Liquid Chromatography*. New York: Wiley.

Detectors: UV/Visible Detection

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The UV absorption detector, introduced in the early 1960s, was the first high sensitivity liquid chromatography detector to be developed. UV absorption detectors are considered to be those detectors that can sense substances that absorb light over the wavelength range 180–350 nm. Many compounds absorb light in this range, including those substances having one or more double bonds (π electrons) and those that have unshared (unbonded) electrons, e.g. all olefins, all aromatics and compounds, for example, containing $>C=O$, $>C=S$ and $-N=N-$ groups. As a result, providing the wavelength of the incident light can be selected, the UV detector tends to perform as a universal detector. There are exceptions: the UV detector will not readily detect hydrocarbons, aliphatic alcohols or other substances that do not have a UV chromophore that will absorb light in the wavelength range already defined.

The sensor cell consists of a short cylindrical tube having two terminating flat quartz windows and radial connections at either end for the column eluent to enter and to leave. To reduce band dispersion, the volume of the cell is usually limited to between 2 and 5 μL. The UV light passes axially through the end windows and falls on a photoelectric cell (or array), the output from which is conveyed to an appropriate amplifier and thence to a recorder or data acquisition system.

The cell must be carefully designed to reduce peak dispersion that would result from the natural parabolic velocity profile of the mobile phase as it passes through the cell. A diagram of a typical UV absorption sensor cell is shown in **Figure 1**. The inlet and outlet conduits are designed to produce secondary flow and break up the parabolic velocity profile that causes peak dispersion. Mobile phase enters the cell at an angle, and is directed at the cell window. As a consequence, the stream of mobile phase must virtually reverse its direction to pass through the cell, producing a strong radial flow which disrupts the Newtonian flow.

The same situation is arranged to occur at the exit end of the cell. The flow along the axis of the cell must reverse its direction to pass out of the port that is set at an angle to accomplish the same effect. By employing this type of cell geometry, dispersion in the cell resulting from viscous flow can be practically eliminated.

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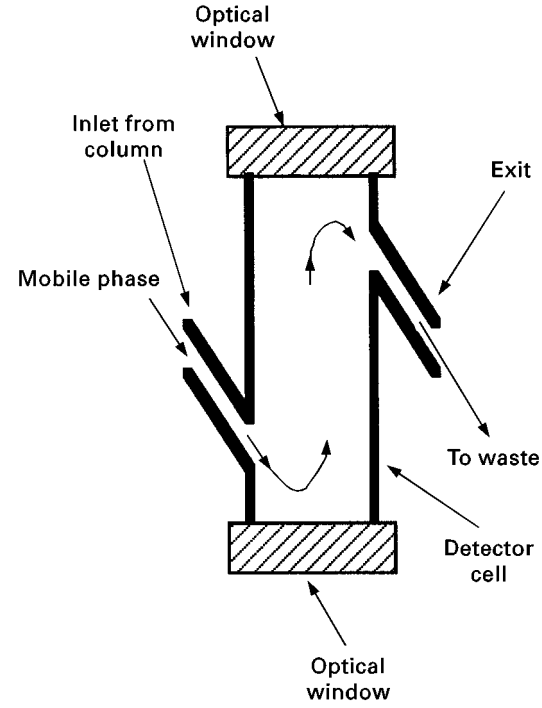

Figure 1 A simple UV detector sensor cell.

The relationship between the intensity of UV light transmitted through a cell (I_T) and the concentration of solute in it (c) is given by Beer's law:

$$I_T = I_0 e^{-\varepsilon lc} \quad \text{or} \quad \ln(I_T) = \ln(I_0) - \varepsilon cl \qquad [1]$$

where I_0 is the intensity of the light entering the cell, l is the path length of the cell, and ε is the molar extinction coefficient of the solute for the specific wavelength of the UV light.

If eqn [1] is put in the form:

$$I_T = I_0 10^{-\varepsilon lc} \qquad [2]$$

The sensitivity of the detector is directly proportional to the value of the absorption coefficient and the path length of the cell. Thus, the sensitivity of the detector can be increased by increasing the path length. However, the volume of the cell must also be constrained to avoid more than a small fraction of a peak existing in the cell at any one time (this can cause peak dispersion and, at the extreme, when two peaks exist in the tube, peak merging). Consequently, as the length is increased, the radius must be reduced, which will increase the detector noise and cause a reduction in sensitivity. Thus, increasing the detector sensitivity by increasing the path length has limitations, and a well-designed cell involves a careful compromise between cell radius and length to provide the maximum sensitivity. Most modern UV detector sensors have path lengths that range between 1 and 10 mm and internal radii that range from about 0.25 to 1 mm.

From eqn [2]:

$$\log \frac{I_T}{I_0} = \varepsilon lc = A \qquad [3]$$

where A is termed the absorbance.

The term ΔA is often employed to define the detector sensitivity where the value of ΔA is the change in absorbance that provides a signal-to-noise ratio of 2, i.e.:

$$\Delta A = \varepsilon l \Delta c \qquad [4]$$

where (Δc) is the detector concentration sensitivity or minimum detectable concentration. Thus:

$$\Delta c = \frac{\Delta A}{l\varepsilon} \qquad [5]$$

It is clear that two detectors having the same sensitivity, defined as ΔA, will not necessarily have the same sensitivity with respect to solute concentration. Two detectors having the same minimum detectable change in absorbance will only exhibit the same concentration sensitivity if the path lengths of the two sensors are identical.

UV detectors can be used with gradient elution providing the solvents do not absorb significantly over the wavelength range that is being used for detection. The solvents employed in reversed-phase chromatography are usually water, methanol, acetonitrile and tetrahydrofuran (THF), all of which are transparent to UV light over the wavelength range commonly used. In normal-phase operation, however, more care must be taken in solvent selection, as many solvents that would be appropriate for the separation absorb UV light very strongly. The *n*-paraffins, dichloromethane, and *n*-paraffins containing small quantities of aliphatic alcohols or THF are useful solvents that are transparent in UV, and can be used with normal distribution systems (e.g. with a polar stationary phase such as silica gel).

There are two types of UV detector: fixed-wavelength detector and multiple-wavelength detector.

Fixed-wavelength Detector

The fixed-wavelength detector functions with light of a single wavelength (or nearly so) generated by a specific type of discharge lamp. The most popular lamp for this purpose is the low pressure mercury vapour lamp, which generates most of its light at 254 nm. There are other lamps that are used with fixed-wavelength UV detectors: the low pressure cadmium lamp which generates light predominantly at 225 nm and the low pressure zinc lamp that emits light at 214 nm. None of the lamps are monochromatic and light of other wavelengths is always present but usually at a significantly lower intensity. The low pressure mercury light source (actual maximum emission wavelength 253.7 nm) is the lamp which is most frequently used in the fixed-wavelength detector.

The typical optical system of a fixed-wavelength UV detector is shown in **Figure 2**. Light from the UV source is collimated by a suitable lens through the sample cell and the reference cell, and then on to two photocells. The cells are cylindrical, with quartz windows at either end. The reference cell compensates for any absorption that the mobile phase might have at the sensing wavelength. The outputs from the two photocells are passed to a signal-modifying amplifier so that the output is linearly related to the concentration of solute being detected.

For reasons already discussed, modern sensor cells have angular conduits that form a Z shape to reduce dispersion. The UV detector can be fairly sensitive

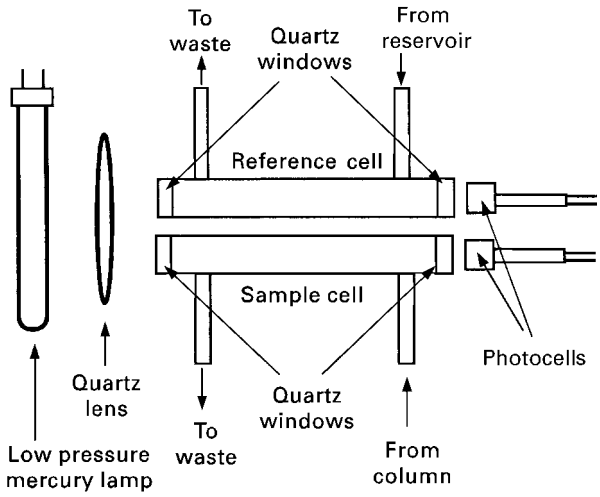

Figure 2 A fixed-wavelength UV detector.

to both flow rate and pressure changes but this instability can be greatly reduced if the sensor is well thermostatted. The fixed wavelength UV detector was once one of the most commonly used LC detectors; it is sensitive, linear and relatively inexpensive. However, today the diode array detector is the most popular, despite its much higher price. The sensitivity (minimum detectable concentration) of the fixed-wavelength detector to compounds with favourable detection properties is about 5×10^{-8} g mL^{-1}, with a linear dynamic range of about three orders of magnitude.

Multiple-wavelength Detector

The multiple-wavelength detector requires a source that emits light over a wide range of wavelengths and, with the aid of a monochromator, light of a specific wavelength can be selected for detection purposes. Detector sensitivity can be improved by selecting light of a wavelength at which the solute has its maximum absorption. Alternatively, the emerging solution peak can be scanned over a range of wavelengths, and the absorption spectra of eluted substances can be used for identification purposes.

There are two basic types of multiple-wavelength detector: the dispersion detector and the diode array detector, the latter being the more popular. In fact, there are currently very few dispersion instruments commercially available but as there are many still in use, their characteristics will be discussed.

The two types of multiple-wavelength detectors have important differences. In the dispersive instrument, the light is dispersed before it enters the sensor cell, and thus virtually monochromatic light passes through the cell. However, if the incident light can excite the solute and cause fluorescence at another wavelength, then the light falling on the photocell will contain that incident light that has been transmitted through the cell, together with any fluorescent light that may have been generated in the cell. Consequently, the light monitored by the photocell will not be solely monochromatic and light of another wavelength, if present, could impair the linear nature of the response. In most cases this effect is negligible, but with some substances it may be quite significant. The diode array detector operates in quite a different manner. Light of all wavelengths generated by the deuterium lamp is transmitted through the cell, and the transmitted light is then dispersed over an array of diodes. In this way, absorption at discrete groups of wavelengths is continuously monitored at each diode. However, the light falling on a discrete diode may not be solely that transmitted through the cell from the source, but may contain fluorescence light excited by light of a shorter wavelength. In this case, the situation is exacerbated by the fact that the cell contents are exposed to all the light emitted from the lamp, and so fluorescence is more likely. In general, this means that, under some circumstances, the transmitted light measured may also contain fluorescent light. As a consequence, the absorption spectrum obtained for a given substance may be degraded from the true absorption curve.

The ideal multiple-wavelength detector would be a combination of both the dispersion instrument and the diode array detector. This system would allow a monochromatic light beam to pass through the detector and then the transmitted beam would itself be dispersed again on to a diode array. Only that diode corresponding to the wavelength of the incident light would be used for monitoring the transmission. In this way any fluorescent light would strike other diodes, the true absorption would be measured and, if the incident light is scanned, accurate absorption spectra could be obtained.

The Multiple-wavelength Dispersive UV Detector

A conventional multiple-wavelength dispersive UV detector is shown in **Figure 3**. Light from the source is collimated by two curved mirrors on to a holographic diffraction grating. The dispersed light is then focused by means of a curved mirror on to a plane mirror and light of a specific wavelength is selected by appropriately positioning the angle of the plane mirror. Light of the selected wavelength is then focused by means of a lens through the flow cell and, consequently, through the column eluent. The exit beam from the cell is then focused by another lens on to a photocell which gives a response that is some func-

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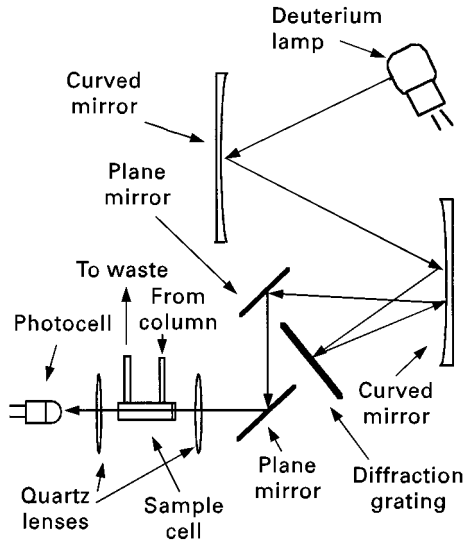

Figure 3 The multiple-wavelength dispersive UV detector. Courtesy of the Perkin Elmer Corporation.

tion of the intensity of the transmitted light. The detector is usually fitted with a scanning facility that, by arresting the flow of mobile phase, allows the spectrum of the solute contained in the cell to be obtained. Due to the limited information given by most UV spectra, and the similarity between UV spectra of widely different types of compounds, the UV spectrum is not usually very reliable for structure elucidation.

UV spectra are, however, useful for determining the homogeneity of a peak by obtaining spectra from a sample on both sides of the peak. The technique is to normalize both spectra, then either subtract one from the other and show that the difference is close to zero, or take the intensity ratio across the peak and show that it is constant throughout the peak.

A common use of the multiple-wavelength detector is to select a wavelength that is characteristically absorbed by a particular component or components of a mixture to enhance the sensitivity of the detector to those particular solutes. Alternatively, by choosing a characteristic absorption wavelength of the solute, the detector response can be made specific to the solute(s) and thus not respond significantly to other substances in the mixture that are of little interest.

The early multiple-wavelength, dispersive UV detector proved extremely useful, providing adequate sensitivity, versatility and a linear response. It was found, however, to be bulky (due to the need for a relatively large internal optical bench), required mechanically operated wavelength selection and a stop/flow procedure to obtain spectra 'on-the-fly'. The alternative diode array detector has all the advantages but none of the disadvantages and, as a result, has become the variable-wavelength UV detector of choice.

The Diode Array Detector

The diode array detector also utilizes a deuterium or xenon lamp that emits light over the UV spectrum range. Light from the lamp is focused by means of an achromatic lens through the sample cell and on to a holographic grating. The dispersed light from the grating is arranged to fall on a linear diode array.

The resolution of the detector ($\Delta\lambda$) will depend on the number of diodes (n) in the array, and also on the range of wavelengths covered ($\lambda_2 - \lambda_1$). Thus:

$$\Delta\lambda = \frac{\lambda_2 - \lambda_1}{n} \qquad [6]$$

It is seen that the ultimate resolving power of the diode array detector will depend on the semiconductor manufacturer and on how narrow the individual photocells can be commercially fabricated.

A diode array detector is shown in **Figure 4**. Light from the broad emission source is collimated by an achromatic lens, so that the total light passes through the detector cell on to a holographic grating. In this way the sample is subjected to light of all wavelengths generated by the lamp. The dispersed light from the grating is then allowed to fall on to a diode array. The array may contain many hundreds of diodes, and the output from each diode is regularly sampled by a computer and stored on a hard disk. At the end of

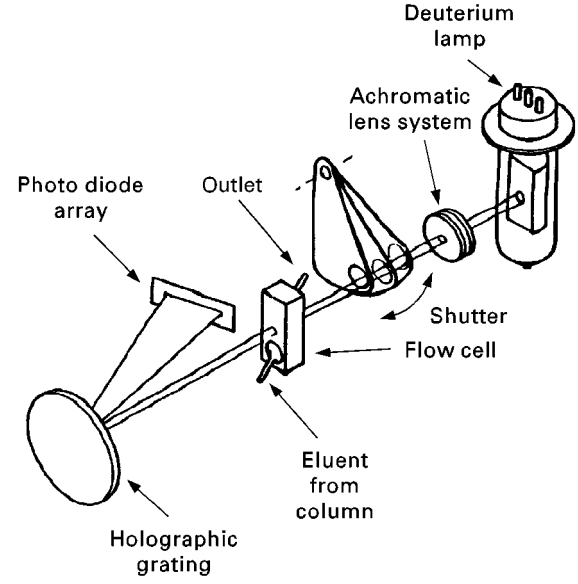

Figure 4 The diode array detector.

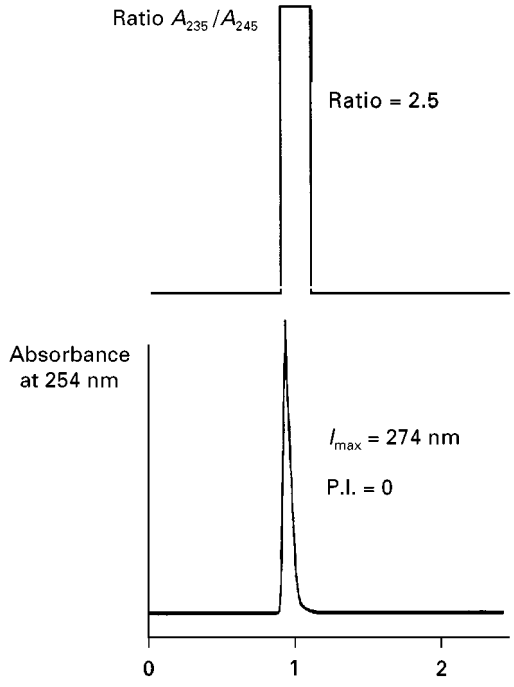

Figure 5 Dual-channel plot from a diode array detector confirming peak purity. Chlorthalidone was isolated from a sample of tablets and separated by a reversed-phase (C_{18}) column 4.6 mm i.d., 3.3 cm long, using a solvent mixture consisting of 35% methanol, 65% aqueous acetic acid solution (water containing 1% acetic acid). Flow rate: 2 mL min^{-1}. Courtesy of the Perkin Elmer Corporation.

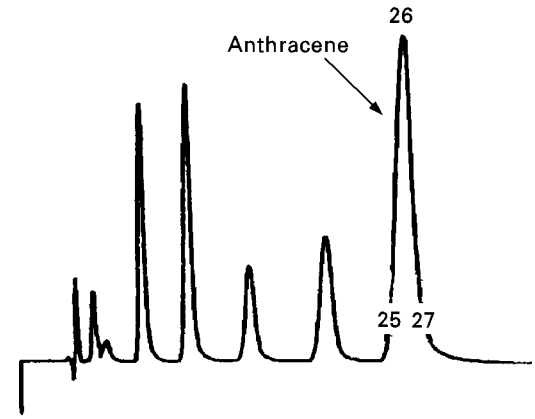

Figure 6 Separation of some aromatic hydrocarbons. Separation was carried out on a 3 cm long, 4.6 mm in diameter column packed with a 3 μm C_{18} sorbent. Courtesy of the Perkin Elmer Corporation.

the run, the output from any diode can be selected and a chromatogram produced using the UV wavelength that was falling on that particular diode.

Most instruments will allow at least one diode to be monitored in real time, so that a chromatogram can be generated as the separation develops. This system is ideal, as by noting the time of a particular peak, a spectrum of the solute can be obtained by recalling from memory the output of all the diodes at that particular time. This directly produces the spectrum of the solute. The diode array detector can be used in a number of unique ways: one example is to use it to verify the purity of a given solute, as shown in **Figure 5**. The chromatogram, monitored at 274 nm, is shown in the lower part of Figure 5. As a diode array detector was employed, it was possible to ratio the output from the detector at different wavelengths and plot the ratio simultaneously with the chromatogram monitored at 274 nm. Now, if the peak were pure and homogeneous, the ratio of the absorption at the two wavelengths (those selected being 225 and 245 nm) would remain constant throughout the elution of the entire peak.

The top part of Figure 5 shows this ratio plotted on the same time scale as the elution curve, and it is seen that a clean rectangular peak is produced, confirming a constant absorption ratio at the two wavelengths. The wavelengths chosen to provide the confirming ratio will depend on the UV absorption characteristics of the substance concerned. Another interesting example of the use of the diode array detector to confirm the integrity of an eluted peak is afforded by the separation of the series of aromatic hydrocarbons shown in **Figure 6**.

It is seen that the separation appears to be satisfactory and, without further evidence, it would be reasonable to assume that all the peaks were pure. However, by plotting the absorption ratio, 250 nm/255 nm, for the anthracene peak it becomes evident that the tail of the peak contains an impurity as the clean rectangular shape of the ratio peak is not realized. The absorption ratio peaks are shown in **Figure 7**.

The presence of an impurity is confirmed unambiguously by the difference in the spectra obtained for

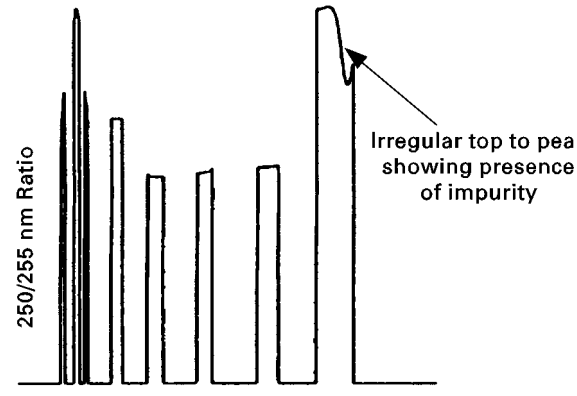

Figure 7 Curves relating the absorption ratio and elution time.

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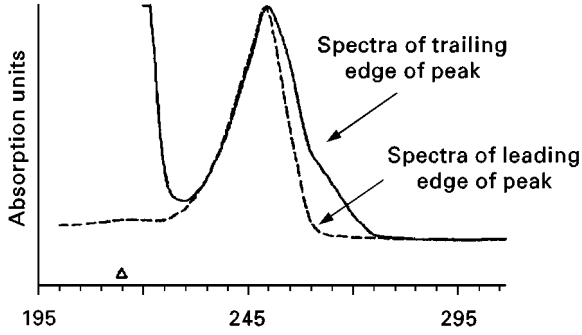

Figure 8 Superimposed spectra taken at the leading and trailing edges of the anthracene peak.

the leading and trailing edges of the peak. Spectra taken at the leading and trailing edge of the anthracene peak are shown superimposed in **Figure 8**. Further work identified the impurity to be 5% t-butylbenzene.

The diode array detector is now generally considered the most versatile and useful detector for everyday use in liquid chromatographic analyses. The performances of both types of multiple-wavelength detectors are very similar and typically have a sensitivity of about 1×10^{-7} g mL^{-1} and a linear dynamic range of about three orders of magnitude: 1×10^{-7} to 5×10^{-4} g mL^{-1}. The device automatically records a spectrum at each sampling point and thus is extremely rapid. It is very suitable for use with fast separations completed in a few seconds.

The use of UV detection in capillary electrochromatography and for detection in capillary electrophoresis has enjoined a novel cell design. As the peaks are only a few nanolitres in volume, the sensor volume must be commensurately small. A practical detector has been constructed by removing the polyamide coating from the surface of a short length of quartz capillary tubing, as shown in **Figure 9**.

UV light from a suitable lamp traverses the tube window and falls on to a photocell. As the solutes migrate across the window they are detected by light absorption in the usual manner. Considering the expression given in eqn [1] for the output of the detector, it appears that, due to the very short pathlength of the cell (*c.* 300 μm), the detector would be very insensitive. However, the loss of sensitivity caused by the reduced pathlength is partly compensated by the relatively high solute concentration in the peaks resulting from the very high column efficiencies achieved in capillary electrochromatography (*c.* 10^5 theoretical plates). The electronic circuitry used with the microcell is basically the same as that used in the conventional larger cell detectors.

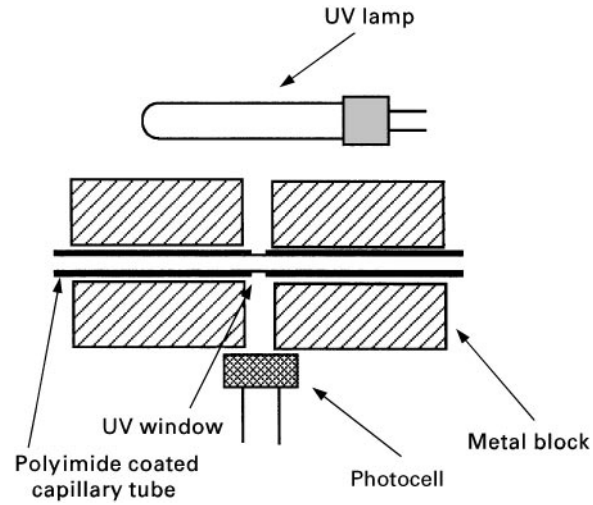

Figure 9 UV detector cell for capillary electrochromatography.

Conclusion

The design of the UV detector has changed little over the last decade and its performance has been only marginally improved. Radical changes do not appear likely in the future, although slow progress in improved sensitivity and cell design can be anticipated. The diode array detector is likely to continue as the 'workhorse' detector for liquid chromatography for many years to come. Further details on liquid chromatography detector design and performance can be found in the Further Reading section.

Further Reading

Katz E and Scott RPW (1982) Liquid chromatography system for fast, accurate analysis. *Journal of Chromatography* 253: 159.

Krull IS (ed.) (1986) *Reaction Detection in Liquid Chromatography*. New York: Marcel Dekker.

Parriott D (ed.) (1993) *A Practical Guide to HPLC Detection*. San Diego: Academic Press.

Scott RPW (1996) *Chromatography Detectors*. New York: Marcel Dekker.

Sharaf MA (1997) Assessment of chromatographic peak purity. *Advances in Chromatography* 37: 1–28.

Vickrey TM (ed.) (1983) *Liquid Chromatography Detectors*. New York: Marcel Dekker.

Yeung ES (ed.) (1986) *Detectors for Liquid Chromatography*. New York: Wiley.